Table 2 Number of alleles and observed heterozygosity values from cross-species amplification within the family Percidae using *Perca flavescens* microsatellite primers. Amplification was attempted on *N* individuals. Number in parentheses are the number of individuals that amplified for each species/locus combination

		Locus									
Species	*N*	*Pfla* L1	*Pfla* L2	*Pfla* L3	*Pfla* L4	*Pfla* L5	*Pfla* L6	*Pfla* L7	*Pfla* L8	*Pfla* L9	*Pfla* L10
Stizostedion canadense	10	7(10) $H = 0.70$	12(10) $H = 1.00$	4(10) $H = 0.30$	—	—	—	—	9(10) $H = 0.70$	—	—
Stizostedion vitreum	10	3(10) $H = 0.20$	2(8) $H = 0.10$	2(10) $H = 0.10$	—	—	—	—	8(10) $H = 0.70$	1(9) $H = 0$	—
Stizostedion lucioperca	8	— $H = 0.38$	3(6) $H = 0$	2(8)	—	—	—	—	4(8) $H = 0.63$	2(7) $H = 0.38$	—
Perca fluviatilis	14	5(14) $H = 0.57$	6(14) $H = 0.36$	—	7(14) $H = 0.71$	6(14) $H = 0.64$	6(14) $H = 0.50$	—	1(13) $H = 0$	8(13) $H = 0.50$	7(14) $H = 0.64$

—, indicates no amplification.

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Isolation of microsatellite markers in the digenetic trematode *Schistosoma mansoni* from Guadeloupe island

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Keywords: genetic variability, microsatellites, Schistosomes

Received 17 November 1999; revision accepted 24 January 2000

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Schistosomes are dioecious blood helminth parasites of human beings and rodents in tropical countries. Development of methods allowing more precise characterization of intraspecific genetic variation and population structure will greatly improve our understanding of schistosome epidemiology and transmission patterns.

In Guadeloupe (French West Indies), numerous foci have been surveyed during several years for the ecology and dynamics of *Schistosoma mansoni* populations among its murine definitive host (*Rattus rattus*) and intermediate mollusc host (*Biomphalaria glabrata*) (Théron & Pointier 1995). Distribution of genetic diversity within and among adult and larval schistosome populations was previously studied using isozymes (Rollinson *et al*. 1986) and RAPD markers (Barral *et al*. 1996; Sire *et al*. 1999). However isozymes loci were not sufficiently polymorphic, and RAPD markers are dominant markers which prevent access to the heterozygosity. The aim of this work was to detect high polymorphic markers such as microsatellites to analyse the fine local structure of infra-populations of schistosomes within and between individual hosts.

After complete digestion of *S. mansoni* DNA with *Sau*3AI, we screened a partial genomic library consisting of about 2894 fragments (size 200–700 bp) ligated into a plasmid pBluescript II SK (+) (Stratagene), digested with *Bam*HI. Colony hybridization was performed with synthetic $(CA)_{10}$ and $(GA)_{10}$ oligonucleotide probes using DIG labelling Kit (Boehringer) according to the protocol of Estoup & Martin (1996). A total of 18 positive clones (0.6% of all colonies screened) were sequenced with [γ-^{33}P] ATP end-labelled with T7 DNA polymerase (Pharmacia) and/or with automatic sequencer (Genome Express). Fifteen sequences containing microsatellites were selected and the primers of corresponding flanking regions defined. In addition, approximately 7000 sequences of *S. mansoni* from EMBL and GenBank databases were checked to detect microsatellites. Eighteen short repeat sequences were selected according to the length of flanking sequences to design primers using OSP version 5.0 software.

Polymerase chain reaction (PCR) was performed in a 40-μL reaction volume containing 30 pmol of each primer, 1 mM dNTPs (Boehringer), 4 μL buffer 10× (10 mM Tris-HCl pH 9.0,

Table 1 Primer sequences and characteristics of *Schistosoma mansoni* microsatellite loci, including locus name, GenBank Accession no., primer sequences, specific annealing temperature (T_a), size of PCR products in base pairs (bp), number of alleles, observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), sample size and repeat array

Locus	Accession no.	Primer sequences (5′–3′)	T_a °C	Size bp	No. of alleles	H_O	H_E	Sample size	Repeat array
SMD25	AF202965	F: GATTCCCAAGATTAATGCC R: GCCATTAGATAATGTACGTG	48	292	3	0.10	0.19	10	$(CA)_{10}$
SMD28	AF202966	F: CATCACCATCAATCACTC R: TATTCACAGTAGTAGGCG	48	240	2	0.08	0.08	12	$(CAA)_5$
SMD57	AF202967	F: TCCTTGATTCCACTGTTG R: GCAGTAATCCGAAAGATTAG	50	297	3	0.40	0.41	10	$(TA)_{22}(GA)_9$
SMD89	AF202968	F: AGACTACTTTCATAGCCC R: TTAAACCGAAGCGAGAAG	51	153	3	0.21	0.28	19	$(TC)_8$
SMD94	AF202969	F: TAACACTCACACATACCC R: AACTAATCACCCACTCTAC	51	184	2	0.06	0.06	17	$(TC)_5$
AI068335	AI068335	F: GTTGAGAGAGAAAAAGAAG R: AGATGTTAGAAAGTGGTG	51	269	2	0.08	0.08	12	$(TG)_{10}$
L46951	L46951	F: CAAACATATACATTGAATACAG R: TGAATTGATGAATGATTGAAG	48	172	2	0.55	0.51	11	$(GAA)_7$
SCMSMOXII	M85305	F: TTCTACAATAATACCATCAAC R: TTTTTTCTCACTCATATACAC	48	295	3	0.60	0.45	10	$(CAT)_9CGT(CAT)_6$
R95529	R95529	F: GTGATTGGGGTGATAAAG R: CATGTTTCTTCAGTGTCC	51	243	4	0.44	0.46	16	$(CAT)_{10}$
SMU31768	U31768	F: TACAACTTCCATCACTTC R: CCATAAGAAAGAAACCAC	48	203	2	0.11	0.53	9	$(GAT)_8$
SMIMP25	X77211	F: CACTATACCTACTACTAATC R: TCGATATACATTGGGAAG	49	219	8	0.90	0.83	19	$(TA)_{16}$

50 mM KCl, 0.1% Triton X-100, Promega), 1.5 mM $MgCl_2$ (Promega), 2 U *Taq* polymerase (Promega) and approximately 20 ng DNA template. The PCR programme consisted of initial denaturation at 94 °C for 4 min, followed by 30 cycles at 94 °C for 30 s, annealing temperature for 30 s (see Table 1 for details), 72 °C for 30 s, and a final extension at 72 °C for 10 min in an MJ-Research PTC100 thermocycler. PCR products were mixed with one third volumes of formamide loading buffer and denatured at 95 °C for 5 min, prior to electrophoresis in a denaturing (7 M urea) 8% Long Ranger (Tebu) polyacrylamide gel (18 × 20 cm, Pharmacia). The PCR products were revealed by silver nitrate (Sigma) staining.

A total of 33 microsatellites loci were used to detect length polymorphism. DNA samples from adult schistosomes from two wild rats *R. rattus* trapped in the Dans-Fond locality (Guadeloupe) were extracted using a 5% Chelex-100 (Bio-Rad) extraction method. Of the 33 microsatellite sequences examined, di-, tri- and tetranucleotide repeats were found and the frequencies of perfect, imperfect, and compound repeats were 61, 30 and 9%, respectively. Eleven loci were polymorphic with the number of alleles ranging from 2 to 8 (Table 1). The observed (H_O) and expected heterozygosities (H_E) varied considerably between loci (Table 1). These microsatellite loci will be highly useful to analyse the mono- vs. pluri-infection within intermediate hosts, the recruitment pattern by the definitive hosts inferred from the amount of parasite genetic variability within and between individual hosts, and the population structures of schistosome at different spatial scales (regional vs. local) in a metapopulation system.

Acknowledgements

This work received financial support from the CNRS (PNDBE) and the MENRT (PRFMMIP). We are grateful to Thierry de Meeûs for a critical reading of this manuscript.

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